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Review

Keep-ING balance: Tumor suppression by epigenetic regulation

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ABSTRACT

Cancer cells accumulate genetic and epigenetic changes that alter gene expression to drive tumorigenesis. Epigenetic silencing of tumor suppressor, cell cycle, differentiation and DNA repair genes contributes to neoplastic transformation.

The ING (inhibitor of growth) proteins (ING1–ING5) have emerged as a versatile family of growth regulators, phospholipid effectors, histone mark sensors and core components of HDAC1/2 – and several HAT chromatin-modifying complexes. This review will describe the characteristic pathways by which ING family proteins differentially affect the Hallmarks of Cancer and highlight the various epigenetic mechanisms by which they regulate gene expression. Finally, we will discuss their potentials as biomarkers and therapeutic targets in epigenetic treatment strategies.

1. Out of balance: epigenetics in cancer

Cancer has been described in terms of ten specific "hallmarks" that are acquired in different combinations by cells undergoing the multistep process of carcinogenesis [1]. These include mutations in tumor suppressor genes and oncogenes, which have stood the test of time as integral culprits of neoplastic transformation. Oncogenesis may also be understood as a cascade of clonal expansions triggered by random attainment of an enabling mutant genotype [2,3]. Continuously increasing evidence indicates that epigenetic alterations also participate with genetic abnormalities, contributing to the hallmarks that drive the process of tumorigenesis [2,4].

The term "epigenetics" was originally coined in the middle of the twentieth century by Conrad Waddington (1905–1975), a British developmental biologist. He linked the two fields of epigenesis and genetics to describe "the causal interactions between genes and their products, which bring the phenotype into being" [5–7]. Despite decades of debate, the definitions of "epigenetics" or "epigenetic" remain somewhat controversial [8]. Most commonly, the term is used to describe reversible, chromatin-based events that regulate DNA-templated processes without altering DNA primary sequence [9]. This definition will be used in this article.

The myriad characteristics of a cellular phenotype that are not due to primary DNA sequence alterations evolve by stable maintenance during differentiation, allowing cells to have distinguishable identities while harboring identical genetic information. This means that gene expression patterns are unique, heritable and mediated by chromatin modifications. One major level of chromatin regulation is at the level of the nucleosome, a simple repeating unit of chromatin that contains about 146 bp of DNA wrapped around an octamer of basic histone proteins. Nucleosomal proteins are subject to modification by phosphorylation, ubiquitination, SUMOylation, methylation, citrullination and acetylation, most of which affect histone turnover and/or nucleosome stability. Along with DNA methylation, histone modification has emerged as a promising target in cancer treatment. Acetylation induced by histone acetyltransferases (HAT) is associated with high transcriptional activity, while histone hypoacetylation induced by histone deacetylases (HDAC) usually results in gene silencing. The epigenetic mechanism that is arguably the best understood currently is the transcriptional repression or activation of a

Abbreviations: bp, base pair; CDK, cyclin-dependent kinase; DNA, desoxyribonucleic acid; DNMT, DNA methyltransferase; H, histone; HAT, histone acetyltransferase; H3K4me3, histone H3 trimethylated on lysine 4; HDAC, histone deacetylase; ING, Inhibitor of growth; mSin3, mammalian SIN3 homolog; *MGMT*, 06-methylguanine-DNA-methyltransferase-gene; NFkB, nuclear factor kappa B; NuA4-Tip60, nucleosome acetyltransferase of H 4-Tat-interactive protein-60; PCNA, proliferating-cell nuclear antigen; PHD, plant homeodomain; PtdIns(5)P, phosphatidylinositol 5-phosphate; siRNA, small interfering ribonucleic acid; SWI–SNF, switching deficient–sucrose non-fermentable; SUMO, small ubiquitin-like modifier; *Rb*, Retinoblastoma-gene; TGFβ, transforming growth factor beta

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growing list of tumor suppressor genes or oncogenes, by altered methylation of DNA in GC-rich promoter regions (CpG islands) of these genes [9–14].

A physiological epigenetic balance is maintained when some specialized genes determining the phenotype of differentiated cells are permanently turned on, while others are permanently turned off. Loss of this balance can result in a cancer epigenome, where aberrant transcription of growth regulatory genes and dysregulated signaling pathways enable emergence of the Hallmarks of Cancer.

In fact, epigenetic imbalance is suspected to be the key initiating mechanism in some forms of cancer [15]. For example, the identification of genes that are specifically hypermethylated (silenced), or hypomethylated (transcriptionally activated), spawned new concepts of cancer initiation, progression and therapy [2,9]. Similar to gene mutations that occur frequently in specific cancers, various high-frequency epigenetic mutations have already been discovered in specific genes. To name a few, the tumor suppressor and growth-regulatory genes Von Hippel–Lindau *(VHL)* in Wilms Tumor, *CDKN2A* (encoding the tumor suppressors INK4A and ARF), *MGMT* (encoding the DNA repair enzyme 0(6)-methylguanine-DNA-methyltransferase), *Rb* as well as the "large tumor suppressor gene 1" *(LATS1)* are hypermethylated in their promoter regions [16–18]. Another mechanism involved in transcriptional and posttranscriptional gene silencing is specific base pairing between microRNAs (miRNAs) and their growth-regulatory targets. miRNAs are vital for normal development and are frequently compromised in diseases such as cancer [19]. Finally, aberrant epigenetic status in cancers is also defined by posttranslational local and global chromatin modifications [2]. Therefore, studying epigenetics in cancer should not only focus on the discovery of growth-regulatory genes *affected,* but also on the recognition of the corresponding culprits: chromatin modifying non-histone proteins, that are dysregulated in cancer cells, such as the ING family of proteins that is the major subject of this review.

In addition to screening cancer-cell genomes and epigenomes for genetic and epigenetic alterations of tumor suppressor genes, cancer patients may profit from the identification and analysis of chromatin-regulatory proteins, since they represent potential biomarkers and targets for epigenetic-based cancer therapies.

2. Almost two decades of learnING: from candidate tumor suppressors to a PHD in epigenetics

The ING (**IN**hibitor of **G**rowth) family of genes and proteins (ING1–ING5) was originally identified in 1996, using subtractive hybridization between cDNAs from a normal mammary epithelial cell strain and several transformed breast cancer cell-lines, followed by an *in vivo* functional screen for tumourigenesis [20]. INGs are evolutionarily well-conserved proteins that localize primarily in the nucleus [21]. Abnormal nuclear morphology is a frequently noted change in cancer cells ever since, more than a century ago, Theodor Boveri described the first genetic alteration in cancer: abnormal chromatin [22,23]. The INGs are multidomain proteins that share motifs targeting them to the nucleus and to different chromatin domains, thereby enabling them to exert their growth-regulatory functions.

2.1. All PHDs: on the histone mark, (de)acetylate, go!

All ING proteins share a *highly conserved plant homeodomain (PHD) finger* (Fig. 1) [21,24,25]. The PHD selectively binds to the lysine 4 residue of histone H3 with affinity increasing with methylation state such that H3K4me3 > H3K4me2 > H3K4me. The H3K4me3 mark is preferentially located at promoters and downstream of transcription start sites [26–34]. It is bound by all ING-PHDs with strong but biologically relevant dissociation constants (*Kds* of <10 μM), and competes for binding with a small number of other PHD-containing proteins. Interaction of INGs with H3K4me3, partially by recruitment of the growth arrest DNA damage protein 45a (Gadd 45a), directs the acetylation and methylation status of histone tails, thereby stimulating or silencing growth-inhibitory or pro-proliferative target gene promoters, in multiple species [29,35–39]. Since all of the ING proteins are stoichiometric members of HAT (ING3–ING5) or HDAC (ING1, ING2) complexes, once they bind the H3K4me3 mark through their PHD, they can direct HAT or HDAC activity to the immediate vicinity of the mark to affect chromatin structure.

Through their conserved PHD, all ING family proteins act as H3K4 methylation-sensitive sensors, thereby transcriptionally regulating target genes. Therefore, they are considered as "readers" of the epigenetic code.

2.2. Nuclear architects sharING motifs

As shown in Fig. 1, ING proteins all encode one or more *nuclear localization signals (NLS)* that are needed for efficient nuclear targeting [40]. Several of the ING proteins also contain short, highly basic *nucleolar translocation sequences (NTS)* within the NLS, which translocate them to the nucleoli in response to DNA damage. Mutation of the NTS results in the loss of stress-induced nucleolar targeting and subsequently reduced levels of apoptosis [41].

Many studies to date reporting on the impact of nuclear structure on gene regulation have been performed using developmental systems or aging models such as the Hutchinson–Gilford Progeria syndrome (HGPS), that has a clear link to disruption of nuclear morphology and gene expression by mutation of the gene encoding lamin A. HGPS is a rare developmental autosomal dominant condition characterized by accelerated aging that begins in early childhood and leads to death at a mean age of 13 years [42]. ING1 is one of the genes that is transcriptionally repressed in HGPS and all ING proteins contain a sequence, the *lamin interaction domain (LID),* that is unique in the entire human proteome. The LID interacts specifically with lamin A and seven other proteins, with lamin A being the highest scoring hit [43]. The interaction between the LID and lamin A is believed to help tether ING(1) in the nucleus, thereby localizing its functions as an epigenetic modifier. Considering that HGPS cells feature aberrant chromatin structure and that ING1 does not interact with the mutant form of lamin A called progerin [43], this raises the possibility that ING proteins contribute to transduction of the HGPS phenotype through altering the epigenetic status of lamin A mutant cells.

Like aging, cancer is associated with global misregulation of gene expression [44–46], suggesting that both aging and cancer are in some aspects, developmental diseases. For example, loss of lamin A due to promoter methylation of the *lamin A/C* gene has been reported for hematologic malignancies [47,48]. While normal B and T cells express lamin A and ING(1), leukemia and lymphoma cells do not, nor do some normal progenitor cells or early B/T cell intermediaries [46,49]. ING2 is overexpressed in colon cancer [50], whereas loss of lamin A is common and a risk factor for

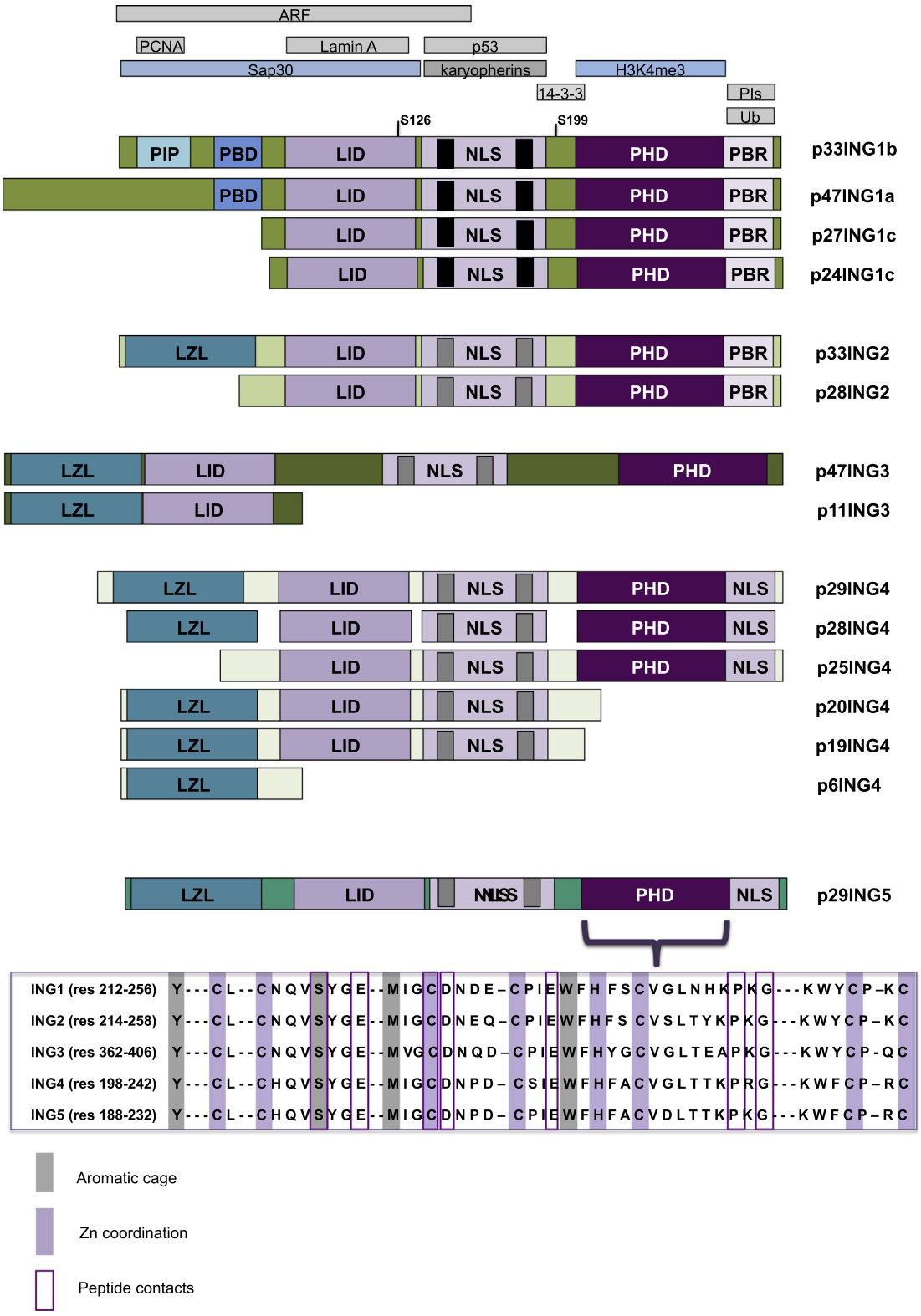

Fig. 1. Nuclear architects with shared and unique domains and motifs: structure of ING family proteins. ING family proteins contain a well conserved plant homeodomain (PHD) and lamin interaction domains (LID) through which they function as H3K4me3 sensors, and bind to Lamin A, respectively. A nuclear localization sequence (NLS) that targets them to the nucleus via karyopherin binding also contains basic nucleolar targeting sequences (NTS, black boxes) but these have only been demonstrated to be active in ING1 and so are indicated in gray in other ING members. The NLS region has also been reported to bind p53. The polybasic region (PBR) binds both bioactive phospholipids (PIs) and ubiquitin (Ub) while ARF and SAP30 have been shown to interact with the amino-terminal half of ING1. Proliferating cell nuclear antigen (PCNA) binds specifically to ING1b via the PCNA-interacting protein (PIP) motif, regulating apoptosis and DNA repair in response to DNA damage. The functions of the partial bromodomain (PBD) still have to be characterized. Two phosphorylation sites located on serines 126 and 199 (S126, S199) have been reported to regulate the half-life of ING1 and alter subcellular localization, respectively. *PHD details (rectangular box):* Amino acid sequences of ING PHD zinc fingers that specifically bind H3K4me3 contain Cys and His residues spaced to coordinate two zinc ions (purple rectangles). The enabling Cys and His residues form an aromatic cage (gray rectangles), while other residues contribute specific hydrogen bonds required for recognizing the H3K4me3 histone mark.

relapse in this cancer type [51]. Thus, if the INGs function as effectors localized by lamin A, colon cancer cells may upregulate ING2 to compensate for lamin A deficiency.

Aging and cancer share many common features of a perturbed nuclear architecture and aberrant gene expression that may be partially due to dysregulated interactions between lamin A and ING proteins.

2.3. Unique motifs for linlNG tumor suppression to transcriptional and post-translational regulation

ING1 also contains a *partial bromodomain (PBD)* [21] (Fig. 1). This motif can interact with SAP30 of the Sin3-HDAC1 and HDAC2 complexes [52], thereby regulating transcription [32,53]. Furthermore, a *polybasic region (PBR)* adjacent to the PHD, is unique for ING1 and ING2 (Fig. 1). Within the human proteome, the ING2 protein shows the strongest affinity for bioactive phosphoinositides such as phosphatidylinositol 5-phosphate (PtdIns(5)P) in the nucleus. This interaction regulates the growth inhibitory effects of ING2 in a p53-sensitive manner [54,55] and is of biological significance for the following reasons: phospholipids that specifically bind to ING1 and ING2 are stress-inducible and highly bioactive. This may explain why ING1 and ING2 proteins targeting HDAC complexes to chromatin are strongly induced in response to stress. Additionally, the PBR overlaps with a *ubiquitin-interaction motif (UIM)* in the C-terminal region of the INGlb isoform [56] (Fig. 1). Ubiquitin and phosphorylated lipid species compete for binding to this site, thereby linking bioactive lipid signaling to ubiquitin-mediated proteasomal degradation [56]. The PBR/UIM of INGlb also interacts with mono-ubiquitinated p53. As a result, the p53 tumor suppressor is stabilized, most likely by blocking polyubiquitination, through targeting deubiquitination by the herpesvirus-associated ubiquitin-specific protease HAUSP [56].

With the exception of ING1, all ING proteins also have a *leucine zipper-like region (LZL)* [21] (Fig. 1) that can help regulate nucleotide-excision-repair-associated functions of ING2, the DNA damage response and cell differentiation [57]. The LZL of ING2 also interacts with components of HDAC1 complexes and it is a critical domain in influencing muscle differentiation by ING2 [57].

The *proliferating-cell nuclear antigen (PCNA)-interacting protein (PIP) is a domain* that is unique to ING1b (Fig. 1). ING1b interacts with PCNA in a UV-inducible manner to initiate DNA repair and/or apoptosis as a stress response [58]. ING1b also contributes to E3 ubiquitin ligase Radl8-mediated PCNA mono-ubiquitination. Mono-ubiquitinated PCNA associates with DNA polymerase η (Polη) to promote proper lesion bypass and error-free DNA replication [59].

Last but not least, recently published results indicate that another domain unique for ING1 and ING2 exists: a *SUMOylation-motif (PDSM),* which provides interaction of ING proteins with the E3-type small ubiquitin-like modifier (SUMO) ligase PIAS4 that has implications in the DNA damage response [60].

Due to their structural characteristics, ING family proteins are nuclear proteins with the ability to act as:

- *histone mark sensors connecting cellular responses to genotoxic stress,*
- *stoichiometric members of HAT and HDAC complexes,*
- *growth regulators.*

2.4. Family meetING

ING family proteins were originally characterized as tumor suppressors based on observations obtained in the era before the development of siRNA technology [61]. Over the last decade, their definition has broadened due to results from multiple siRNA studies and in vivo knockout models. These revealed that ING family proteins differentially control cell growth as epigenetic regulators in different biological contexts.

2.5. ING1 – ImprovING with age

The founding member of the ING family, *ING1*, is localized on chromosome 13q34 [21]. The major ING1-isoforms, ING1a and ING1b, are widely expressed in normal and cancer cells [62,63]. They differ in their N-termini (Fig. 1) and growth-regulatory effects, with ING1a affecting cell senescence [64,65] and ING1b contributing to apoptosis [54,58,66–69]. Over almost two decades of study, ING1 was initially identified as a type-II tumor suppressor, then also as an inducer of replicative senescence and more recently as a transcriptional regulator.

2.5.1. ING1 in human cancers

ING1 gene and/or protein expression is down-regulated in multiple malignancies. These include childhood acute lymphoblastic leukemia [49], neuroblastoma [70], melanoma [71–74], lung [75–78], ovarian cancer [75,78], malignant glioma [79,80], gastric [81], colorectal [82], head and neck squamous cell carcinoma (HNSCC) [83,84], pancreatic [75,85], prostate [75], and breast cancer [75,86,87]. ING1 has also been identified as a breast cancer antigen by serological analysis of recombinant tumor cDNA expression libraries [88]. ING1-overexpression correlated with inhibition of metastasis and reduced tumor-induced mortality of breast cancer *in vivo* [89]. It was also associated with poor prognosis in neuroblastoma [63] and bladder cancer [90]. However, most of these reports do not include information on ING1's subcellular localization. Since ING proteins, like p53 [91], have both nuclear and cytoplasmic functions, localization of ING1 should be confirmed before establishing correlations between expression levels and clinical data.

2.5.2. Mechanisms of ING1 regulation

Ing1-deficient mice are characterized by reduced body size, as well as earlier onset and higher incidence of lymphomas [66,67,92–94]. Moreover, high levels of ING1 were observed in regions of mouse embryos, which are known for increased apoptosis during embryogenesis [95]. Thus, *ING1* downregulation in human cancers may simply reflect a less differentiated phenotype. Alternatively, ING1 loss could itself contribute to the neoplastic process. *ING1*-downregulation was not due to mutation, but frequently correlated with reduced ING1 protein levels [70–87]. These observations are indicative of epigenetic mechanisms regulating *ING1* transcription. *ING1* is very GC-rich in four regions that make it a prime target for CpG-island methylation [96,97]. Indeed, promoter methylation has been reported for *ING1* in ovarian cancers [75] and chronic lymphoblastic leukemia [98]. Abnormal methylation patterns in other genes can also result in *ING1* downregulation, as it has been shown for murine adult brain cells [99]. Intriguingly, the microRNA miR-622 is, like ING1 [81], downregulated and involved in invasion and metastasis of gastric cancer [100]. Moreover, upregulation of miR-622 repressed *ING1*-transcription in this cancer type [100]. Post-translationally, various growth-inhibitory effects of ING1 are regulated by phosphorylation at Ser126 [101,102]. Also, phospho-serine-mediated 14-3-3 binding and nuclear export regulate the ability of ING1b to transcriptionally regulate the p21

cyclin-dependent kinase inhibitor and subsequent induction of apoptosis in response to DNA damage [103]. Moreover, the proto-oncogene Src, a non-receptor tyrosine kinase with an important role in growth factor signal transduction, phosphorylates ING1b, relocalizes it to the cytoplasm, thereby regulating its pro-apoptotic abilities [104]. Overall, valid interpretation of *ING1* downregulation by cancers requires more studies of *ING1* promoter methylation status, of subcellular ING1 protein localization as well as co-expression analyses of other growth-regulators, including additional miRNAs, of which ING1 may be a *bonafide* target.

ING1 is downregulated but not mutated in various human cancers. ING1 expression is regulated by epigenetic mechanisms, such as promoter methylation, miR-622 and by PTMs such as stress-inducible phosphorylation by Src.

2.5.3. Affecting cancer hallmarks by epigenetic regulation

Overexpression of human ING1 causes cell-cycle arrest in the G_0/G_1-phase, whereas suppression leads to loss of cellular growth control and immortalisation [20]. While ING1b is mainly involved in regulating apoptosis, cellular stress, hormonal responses as well as inhibition of tumor angiogenesis [105–107], ING1a inhibits growth by inducing replicative senescence, partially through inhibiting endocytosis via the Rb-tumor-suppressor pathway [64,65]. ING1b can efficiently induce apoptosis through interaction with other tumor suppressors like p53 [68,108,109] and with members of the intrinsic apoptotic pathway such as BCL-2-associated X protein (BAX) [69]. Moreover, Ingl inhibits proliferation of mature B cells in mice [110]. In *Ing1* knockout mice, however, loss of p37Ing1 induces Bax expression and promotes DNA damage-induced apoptosis in a p53-independent manner [110]. *Ing1*-loss also increases sensitivity to ionizing radiation and is associated with earlier onset and higher incidence of lymphomas in mice [110]. This may be due to the involvement of Ing1 in regulating various DNA repair mechanisms in response to stress [111]. These observations suggest both tumor suppressive and oncogenic effects of ING1, possibly depending on the species and biological context.

Major Hallmarks of Cancer affected by ING1 (Fig. 2):

- dysregulated growth suppression,
- replicative immortality,
- invasion and metastasis,
- resistance to cell death,
- aberrant proliferative signaling,
- pathological angiogenesis.

This potentially dual function of ING1 may be due to its function as an epigenetic regulator. ING1 can both activate and repress target genes, since it is an H3K4me3 sensor that

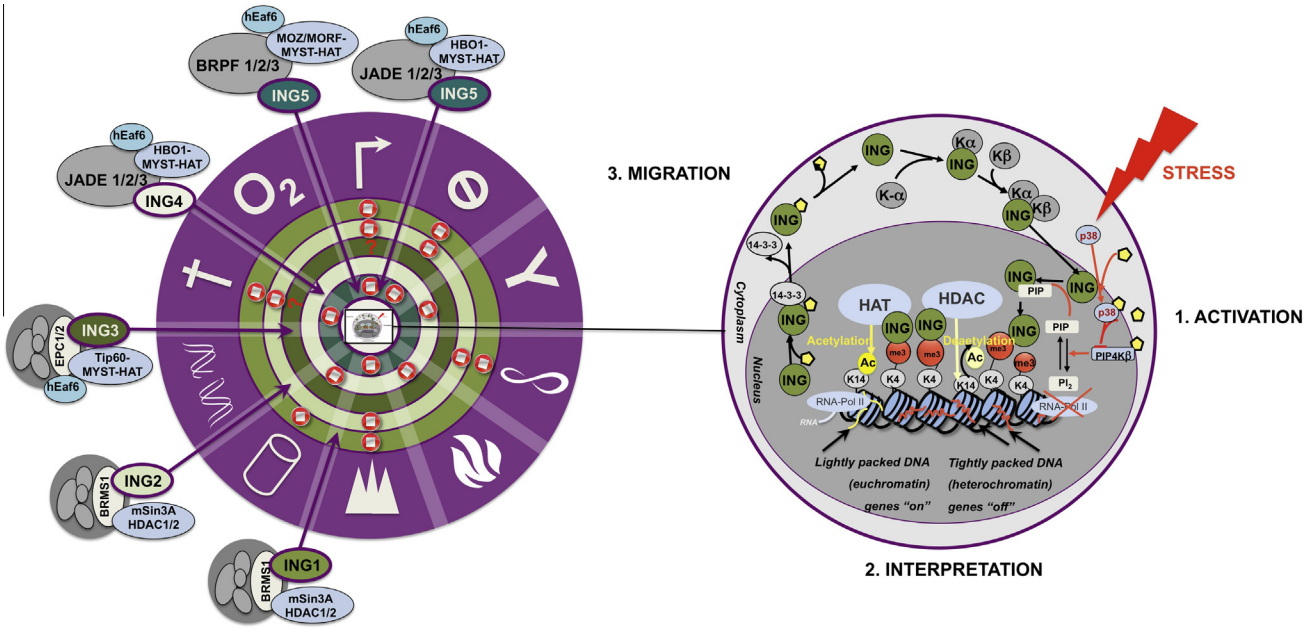

Fig. 2. Left panel: hallmarks of cancer affected by ING-HDAC- and HAT-complexes. *White shapes and symbols clockwise (according to Hanahan and Weinberg 2011) [1]*: arrow: aberrant proliferative signaling, "No"-symbol: dysregulated growth suppression, Y: immune destruction, ∞ : replicative immortality, flame: tumor-promoting inflammation, triangles: invasion & metastasis, pipe: pathological angiogenesis, DNA: genome instability & mutation, cross: resistance to cell death, "O_2": deregulated cellular energetics, red "stop"-sign: inhibited by ING activity, question-mark: possibly inhibited by ING(5) activity. *Abbreviations:* HAT – histone acetyl transferase, HBO1 – HAT of the MYST family, HDAC1/2 – histone deacetylase 1/2, ING (1–5) – Inhibitor of growth (1–5), JADE – zinc finger protein associating with MYST-HATs, mSin3A – core component of large multiprotein HDAC-associated corepressor complex, Tip60-MYST – HAT of the MYST family. *Subunits/domains not explained in the text:* BRMS1 – breast cancer metastasis suppressor 1, BRPF1/2/3 -PHD proteins bridging MOZ and MORF in MYST-HAT-complexes, EPC1/2 -ectopically parting cells 1/2, hEaf6 – subunit of MYST-HAT-complexes. *For detailed information on proteins and signaling pathways associated with the tumor suppressive functions of ING proteins*: see main text. **Right panel:** *Stress signaling and chromatin modification by ING family proteins.* **(1) *Activation:*** upon cellular stress, p38MAPK is activated and then translocated to the nucleus where it phosphorylates PIP4Kβ, thereby inhibiting its kinase activity and increasing nuclear PIP levels. PIP subsequently binds to ING1 and ING2. Recognition of the histone mark H3K4me3 by ING proteins results in formation of ING-HDAC or -HAT complexes. **(2) *Interpretation:*** ING proteins specifically bind to H3K4me3 or H3K14me3 recruiting the assigned chromatin remodeling complexes to specific chromatin loci and target gene promoters (details in main text). This results in various distinc histone-PTMs, such as acetylation, deacetylation and methylation as well as subsequent activation (gene "on") or silencing (gene "off") of gene transcription affecting the Hallmarks of Cancer [1]. **(3) *Migration:*** after realization of histone modifications by ING-HDAC and HAT-complexes, ING proteins are removed and/or inactivated by different PTMs [24]. These include phosphorylation-dependent binding to 14-3-3 proteins leading to cytoplasmatic translocation, whereas dephosphorylation releases ING from 14-3-3, thereby promoting ING binding to karyopherin proteins kα or kβ and subsequent re-localization of ING to the nucleus. *Abbreviations and symbols:* ac – acetyl group, me3 – 3-methyl group, HAT – histone acetyl transferase, HDAC – histone deacetylase, ING – Inhibitor of growth, K4, K14 – core histone subunits, PI – phosphatidylinositol, PIP4Kβ – phosphatidylinositol-5-P 4-kinase type 2 beta, PIP – phosphatidylinositol monophosphate, PTM – post-translational modification, RNA-PolII – RNA polymerase II; yellow pentagons: phosphorylized sites.

recruits mSin3A/HDAC1/2 [52] (Fig. 2). It also cooperates with numerous proteins that are important for different epigenetic modulations, including the Ras, p300 and pl6(INK4a) as well as DNA methyltransferase 1 associated protein (DMAP1) [112–116]. Gadd45a requires specific ING1 binding to H3K4me3 for DNA demethylation [38], thereby linking histone methylation to DNA demethylation. Moreover, ING1 is a transcriptional regulator of multiple miRNAs: INGlb overexpression induces miR-203, miR-375, miR-449b and miR-200c, thereby downregulating Src and CDK6, to name a few, and promoting growth inhibition effects [117]. In mouse primary embryonic fibroblasts, Ing1 regulates the microRNA regulator protein Dgcr8, a protein involved in the early steps of microRNA synthesis [118]. These findings establish a new link between tumor suppression and microRNA biogenesis.

ING1 regulates target gene expression by

- recruitment of protein complexes with chromatin-modifying activity to specific (H3K4me3-marked) promoters,
- regulating Gadd45a-mediated DNA demethylation,
- regulating miRNA expression signatures and biogenesis.

2.6. ING2: The Renaissance Protein

ING2 has high amino acid sequence homology to ING1 [21,119,120] (Fig. 1). Two major splicing variants have been identified for *ING2* (ING2a, ING2b) [121], with the original "ING2" now called ING2a, when required. ING2b lacks the N-terminus that determines major functions of ING2a [122]. ING2 is characterized by its versatility: it is another histone mark sensor that can act as a DNA damage responder and phospholipid signaling effector [123]. In addition to its growth-regulatory abilities, ING2 possesses essential developmental functions, particularly in spermatogenesis [93], axon growth, neuronal and muscle differentiation that are mediated by p53- and chromatin-mediated mechanisms [124], as well as by SnoN-induced transcriptional regulation through ING2 [125].

2.6.1. ING2 in human cancers

Expression of *ING2* is upregulated in colorectal cancer [50,120], in Burkitt lymphoma and cervical cancer according to the Oncomine Database (http://www.oncomine.org/main/login.jsp). Decreased *ING2* expression or loss of heterocygosity have been found in various types of malignancies as well. These include hepatocellular carcinoma (HCC) [126,127], HNSCC [128] and lung cancer [75,129,130]. The subsequent conclusion that ING2 is generally downregulated in these cancers still needs to be confirmed for the following reasons: most of the associated studies were carried out using either a relatively small number of cell-lines [75], or revealed reduced expression of *ING2* at the mRNA level, but no significant changes at the protein level [126]. Despite this, and although subcellular localization of ING2 was cytoplasmatic, even in normal control tissues, ING2 protein expression correlated with unfavorable prognosis [126]. However, ING2 is a nuclear protein [122]. This, as well as additional controls to verify antibody staining specificity should be considered before using expression data in Kaplan–Meier analyses.

2.6.2. Mechanisms of ING2 regulation

Aberrant ING2 levels in cancers appear not to be due to mutations. Instead, direct binding of the p53 tumor suppressor to the promoter region of *ING2* results in ING2 downregulation [131]. Considering that about 50% of sporadic cancers are characterized by *TP53* mutations [132], the upregulation observed for ING2 in colorectal cancers might be, as outlined above, a feedback mechanism due to lamin A loss [51] and/or p53-deficiency. On the other hand, Nutlin 3a, an MDM2 inhibitor that activates p53, is known to downregulate endogenous ING2 levels through binding to two p53 binding sites on the *ING2* promoter. This interaction causes senescence in normal cells [131] and could be a mechanism by which ING2 levels are being reduced in the remaining other 50% of malignancies with wild-type p53. Hence, interpretation of ING2 levels in tumors should also include their p53-status. *ING2*-upregulation may also be a result of direct binding of the transcription factor nuclear factor kappa B (NF-κB) to the *ING2* promoter [133]. NF-κB is antiapoptotic and, like ING2, is overexpressed in colorectal cancer [133]. ING2 protein levels are also regulated by PTMs, and in particular ubiquitin- and SUMO-mediated mechanisms. ING2 interacts with ubiquitin ligase Smad ubiquitination regulatory factor 1 (Smurf1) that targets ING2 for poly-ubiquitination and proteasomal degradation [134], and is regulated by SUMOylation, affecting Sin3A activity [135].

ING2 is differentially expressed in different cancers, transcriptionally repressed by Nutlin3a-mediated p53-activity and activated by NF-κB.

ING2 proteins are post-translationally modified by Smurf-mediated ubiquitin-dependent proteasomal degradation.

2.6.3. Affecting Cancer Hallmarks by epigenetic regulation

ING2 is a damage-inducible gene. The ING2a isoform negatively regulates cell proliferation through p53 activation via acetylation mediated by the HAT p300 [136] and by regulating p21 independently of p53 [137]. ING2 also mediates transforming growth factor-β (TGF-β)-dependent responses in epithelial cells. Considering that TGF-β has growth-inhibitory functions in normal cells and oncogenic functions in invasive metastatic malignancies, the signals from TGF-β mediated by ING2 appear to differ in normal and cancer tissue [138,139]. Moreover, ING2a is required for the initial DNA damage sensing and chromatin regulation in the nucleotide excision repair process via recruitment of the damage-recognition *Xeroderma pigmentosum* group A-complementing (XPA) protein to the lesion site after DNA damage by UV [140]. In contrast, ING2b alone appears not to have any growth-regulatory effects [122]. Thus, the two isoforms may have compensatory roles that protect cells from cell cycle arrest and apoptosis, as only loss of both can induce these biological functions, particularly in p53-deficient cells. These observations imply a role for ING2 proteins in the development of resistance to conventional cancer treatments, such as DNA damaging agents. Moreover, differential regulation of the two ING2 isoforms may be essential for maintenance of irreversible cellular senescence seen following the induction of high levels of DNA damage.

Major Hallmarks of Cancer affected by ING2 (Fig. 2):

- dysregulated growth suppression,
- replicative immortality,
- invasion and metastasis,
- resistance to cell death,
- aberrant proliferative signaling.

Loss of Ing2 in *Ing2*-knockout mice resulted in male sterility and increased incidence of soft tissue sarcomas [124]. In contrast, ING2 loss in human gastric cancer cells induced G_0/G_1 arrest and

reduced their invasive behavior [141]. ING2 appears to have, similar to ING1, differential effects in mice and men, in normal and in cancer cells – a role that is consistent with an epigenetic regulator. Moreover, the PHD finger of ING2 integrates phosphoinositide and chromatin signaling networks to prevent unchecked cell growth: Upon DNA damage, ING2 binds to H3K4me3 [142,143] mediated by nuclear PtdIns(5)Ps, thereby recruiting mSin3a/HDAC1 [144] (Fig. 2). Subsequent promoter binding of the ING2-SIN3A-HDAC1-complex is regulated by SUMOylation of ING2 [135]. To date, more than 200 gene promoters have been identified that are repressed or activated by ING2 in response to genotoxic stress [146]. For example, ING2 silences the candidate proto-oncogene *CIP2A [KIAA1524]* [144], whereas transcription of *matrix metalloproteinase 13 (MMP13)* can be activated or repressed by ING2, thereby regulating the invasion ability of cancers [133,145]. ING2 also binds to the p21 promoter to control the G_1/S checkpoint [139]. It regulates cell cycle progression by blocking the function of RBP1: RBP1 allows recruitment of the mSin3A-HDAC complex by Rb pocket proteins to induce cell cycle arrest through repression of E2F-dependent transcription and DNA replication origins [146]. RBP1-associated mSin3A-HDACl activity is inhibited by SIRT1, which is recruited by ING1 and ING2 proteins [147]. In addition to regulating gene transcription by HDAC- and HAT crosstalk, ING2 collaborates with the tumor suppressive SWI–SNF–BRG1-complex. This is similar to ING1 interacting with the corepressor Alien in chromatin remodeling and both recruit histone methyltransferase (HMT) activity to methylate histone H3 [53,138,148,149].

ING2 regulates target gene expression by

- stress-inducible, phosphoinositide-mediated recognition of H3K4me3, subsequent recruitment of the mSin3A/HDAC1 complex and binding of target gene promoters,
- chromatin remodeling via association with SWI/SNF/BRG1,
- methylation of histone H3 through HMT recruitment.

2.7. ING3 likes MYSTerious HATs

ING3 was identified through bioinformatic analyses of the human genome [62,150]. Its amino acid sequence is the most distinctive among the five ING proteins evolutionarily (Fig. 1) [21]. ING3 modulates cell growth and p53-mediated transcription [150,151]. Although *ING3* is ubiquitously expressed in mammalian tissues, it has been reported that it is more highly expressed in oocytes from mice, rhesus monkeys, and humans [152]. Moreover, ING3 has the highest molecular weight in the family due to long unique, and still uncharacterized regions. These imply unique roles for this family member, such as linking developmental functions to chromatin structure, as recently found in germ and blood cells [152–155].

2.7.1. ING3 in human cancer

Various studies of cancer samples and cell lines analyzed for *ING3* mutation and expression have reported that the *ING3* gene was silenced. These include HNSCC [156,157], HCC [158,159] and ovarian cancer [75].

2.7.2. Mechanisms of ING3 regulation

ING3 is a miR-21 target gene with a role in monocyte differentiation to dendritic cells [155]. Additionally, *ING3* represents a novel transcription target in the serine/threonin kinase RSK2-cAMP-response element-binding protein (CREB) pathway: RSK2 signaling through CREB downregulates ING3 to protect HNSCC cells from the apoptotic "anoikis" process, thereby contributing to cancer cell invasion and tumor metastasis [160]. On the protein level, ING3 is acetylated on several lysine residues that are all located in the unique region of the protein [161]. Thus, ING3 might be distinctively regulated by acetylation. Like ING1 and ING2, ING3 is also ubiquitinated leading to proteasomal degradation [102,162].

ING3 is downregulated in human cancers and transcriptionally regulated by miR-21 and RSK2 signaling through CREB.

ING3 is post-translationally modified by acetylation and ubiquitination at Lys residues within its unique regions.

2.7.3. Affecting Cancer Hallmarks by epigenetic regulation

Numerous reports consider ING3 as another tumor suppressor in the family, since it promotes apoptosis in human cancer cells [148,150]. However, these observations mostly originate from unphysiological overexpression experiments and may require confirmation by knockdown models. In *Caenorhabditis elegans,* however, *ing-3* downregulation by siRNA resulted in strong suppression of stress-induced programmed cell death in germline cells and in the developing embryo [153].

Hallmarks of Cancer potentially affected by ING3 (Fig. 2).

- resistance to cell death,
- aberrant proliferative signaling.

The epigenetic acitivities of ING3 support its potential role as a growth inhibitor in humans: In addition to binding to H3K4me3 similarly to the other ING family members [148], ING3 is a member of the nucleosome acetyltransferase of H4-Tat-interactive protein-60 (NuA4-Tip60)-MYST-HAT complex that acetylates the N-terminal tails of histones H4 and H2A [142,143,148,163] (Fig. 2). The MYST proteins are part of large conserved multisubunit HAT complexes that have diverse roles in gene expression, carcinogenesis, tumor progression, DNA replication and DNA repair [164]. Because the NuA4-Tip60 complex is a MYST-HAT complex that acetylates histones H4 and H2A [165], ING3 may bind to trimethylated lysines of these histones as well. Since histone acetylation and H3K4me3 recognition associate with transcriptional activation, ING3 may be a transcriptional activator only, unlike ING1 and ING2, which can be involved in both transcriptional activation and suppression. Genes regulated by ING3 have yet to be reported.

ING3 is a histone mark (H3K4me3) sensor and member of the NuA4-Tip60-MYST-ING-HAT complex that may activate target genes by histone H2A and H4 acetylation.

2.8. ING4 – that's not old HAT but a HAT-trick!

ING4 was identified by computational homology search [62]. Several alternative transcripts of ING4 have been found so far [166,167] (Fig. 1). One of them (ING4_vs4) was initially designated an *ING4* mutation unique for cancer cells until it was recognized as a splice variant [168]. All ING4 isoforms are ubiquitously expressed in various tissues [166]. The roles of each variant still have to be elucidated, however, designing a specific siRNA against each variant remains challenging. ING4_v2, ING4_v3, and ING4_v4 have 1, 3, and 9 amino acids deleted in the NLS, which are important for

p53 binding [169]. Reports on the effects of small deletions in the NLS on subcellular localization are controversial [166,165,170]. Thus, ING4 isoforms may distribute differentially in different cell types and different biological contexts, and interact with different binding partners to transduce different activities of ING4 during carcinogenesis.

2.8.1. ING4 in human cancers

ING4 is mainly lost or downregulated at the RNA level in human cancers. These include astrocytic tumors of the central nervous system [171,172], breast cancer [75,168,173–175], malignancies of the gastrointestinal tract [176–178], HNSCC [179,180], HCC [181,182], melanoma [75,183], lung [75], ovarian [75] and prostate cancer [75]. Like ING1 [80], ING4 downregulation correlates with higher WHO grades of malignancy in astrocytoma [172], with unfavorable prognosis of breast cancer patients [89,173,184] and with angiogenesis in multiple cancer types [106,184–190] and other diseases [191].

2.8.2. Mechanisms of ING4 regulation

ING4 is downregulated by miR-650 or miR-214, respectively, in leukemia, HCC, lung, gastric and pancreatic cancer [186,192–194], thereby possibly contributing to resistance to apoptosis as well as to certain anticancer agents [195]. ING4 protein stability and activity are primarily regulated post-translationally by citrullination on the NLS-motif. ING4 citrullination alters ING4 half-life, reduces its ability to acetylate p53 and promotes ING4 degradation [196].

ING4 is downregulated in human cancers and transcriptionally regulated by miR-214 and miR-650. ING4 is post-translationally modified by citrullination on the NLS-motif.

2.8.3. Affecting Cancer Hallmarks by epigenetic regulation

In concert with its multiple binding partners such as p53, the HAT p300, HPH-2, which regulates HIF-α stability, the p65 subunit of NF-κB, and liprin α1 [61,197], ING4 primarily acts as a tumor suppressor. Exogenous ING4 reduces cell colony formation, decreases S-phase in cycling cells [138,168], and induces cell death in cancer cells by autophagy [197] or p53-mediated apoptosis [198]. *Ing4*-null mice, however, are viable, develop normally and do not form spontaneous tumors [199]. But *in vivo* models also revealed that Ing4 plays a major role in the inflammatory response of mice to bacterial components through its ability to suppress NF-κB activation of select cytokine genes in stimulated macrophages [199]. Collectively, these results suggest a previously unexplored role also for the human counterpart ING4 in negatively regulating NF-κB-mediated innate immunity. Taken together, ING4 appears to particularly target the three cancer hallmarks angiogenesis, immune destruction and tumor promoting inflammation [1](Fig. 2).

Major Cancer Hallmarks affected by ING4 (Fig. 2):

- immune destruction,
- tumor promoting inflammation,
- angiogenesis.

In addition, ING4 binds to H3K4me3 [200] to associate with the MYST-HB01-JADE-hEAF6-HAT-complex [148,201] (Fig. 2). This latter complex is responsible for most nucleosomal histone H4 acetylation in eukaryotes, and knockdown experiments indicated that Ing4-HB01 association is required for cells to progress properly through the S-phase of the cell cycle [165]. Moreover, ING4 bridging HBO1 with JADE proteins promotes transcriptional activation of erythroid developmental target genes [202]. Although both the ING3 complex and the ING4 complex include MYST-HATs, these acetylases are different between the complexes: the ING3 complex includes Tip60 and the ING4 complex includes HBO1 as a catalytic subunit. The ING4-HB01 complex acetylates histone H4K16, whereas the Tip60-ING3 complex does not acetylate this histone residue [165]. These findings indicate that each ING family protein has a different elaborate role in transcriptional regulation through generation of different "histone code" signals.

ING4 is a histone mark sensor and a member of the HB01-JADE-hEAF6 MYST-ING HAT-complex, thereby activating target genes by acetylation of histone H4.

2.9. Lift two HATs to ING5!

ING5 was identified by computational homology search and shares high amino acid sequence homology with ING4 (Fig. 1) [62,198] ING5 associates with HAT complexes and, depending on the biological context, may have tumor suppressive or oncogenic abilities (Fig. 2).

2.9.1. ING5 in human cancers

A few lung, pancreatic and ovarian cancer cell lines [75], but also bone marrow samples from a large cohort of patients with acute myeloid leukemia (AML) revealed *ING5* downregulation, hinting at a potential tumor suppressive effect of ING5 in these malignancies [203]. In breast cancer, however, only 2/9 cell lines showed aberrant, i.e. deresed *ING5* gene expression [75]. In the majority of gastric cancer samples, ING5 is downregulated at the RNA-, and upregulated on the protein level [204], which may be due to posttranslational dysregulation. *ING5* is also downregulated in HNSCC, in which it has been identified as a tumor suppressor [205,206]. Moreover, cytoplasmic localization of ING5 was significantly increased in these tumors and inversely correlated with nuclear ING5 levels that predict a well-differentiated status. Nuclear localization of ING5 positively correlated with p21 and p300 expression, and with the apoptotic index, suggesting that subcellular mislocalization of ING5 may modulate the transactivation of target genes, thereby compromising apoptosis and cell cycle arrest.

In contrast to the cases noted above, *ING5* gene expression is upregulated in colon cancer [61], suggesting an oncogenic function of ING5 in these malignancies. However, these findings might also reflect that the subcellular localization of ING5, which was not part of this analysis, is of major relevance for its growth regulatory effects: a subsequent study [207] revealed no aberrant *ING5* gene expression, but upregulated protein levels in more than 70% of patients samples and colon cancer cell lines. Moreover, nuclear ING5 negatively correlated and cytoplasmatic ING5 positively correlated with aggressive behavior of the tumors. Thus, both information on expression and subcellular localization of ING5 is required for assessing its role in the biology of colon, and most certainly other cancers. Moreover, expression of REIC [208], a member of the Dickkopf (Dkk) family, might be of relevance when interpreting ING5 expression in colon cancers; REIC/Dkk-3 acts as a tumor suppressor in multiple cancer cell lines by inducing apoptosis through endoplasmic reticulum stress signaling, and significantly negatively correlated with ING5 expression in colon cancers [208]. However, further analysis is needed before establishing whether a functional ING5-Dickkopf interaction exists.

2.9.2. Mechanisms of ING5 regulation

In pancreatic cancer, *ING5* is negatively regulated by miR-196a, upregulation of which is associated with poor prognosis [209]. In mesenchymal stem cells (MSC), ING5 mRNA and protein levels are regulated by miR-193 to control CDK2-mediated MSC proliferation [210]. ING5 protein activity is further controlled by methylation [211] and acetylation [161].

ING5 is differentially expressed in different cancers and transcriptionally regulated by miR-196a. ING5 proteins are post-translationally modified by methylation and acetylation.

2.9.3. Affecting cancer hallmarks by epigenetic regulation

Being a genuine ING, ING5 controls growth differently in different biological settings: ING5 induces cell cycle arrest and apoptosis in normal and cancer cells [148,198,203,206,209]. In murine mesenchymal fibroblasts, Ing5 requires Inhibitor of cyclin Al (INCA1) to promote Fas-induced apoptosis [207]. ING5 is also involved in p53-dependent stress-signaling, partially by physically interacting with p53, p300/CBP- and MYST-HATs (Fig. 2), such as HBO1 and Tip60, thereby inducing the activation of p53-downstream effectors [148,198,212]. ING5 also associates with minichromosome maintenance (MCM) proteins. These play an essential role in DNA replication [148]. Moreover, knockdown of *ING5* completely inhibited DNA synthesis, whereas knockdown of the HAT HBO1 increased cells in S-phase. Thus, the HBOl-JADE-ING5-HAT-complex may be involved in carcinogenesis by enhancing DNA replication. In normal cells, *ING5* inhibition by siRNA resulted in increased MSC proliferation [210], and was found to be part of a network of epigenetic modifiers that regulate epidermal stem cell differentiation [213].

Major Hallmarks of Cancer affected by ING5 (Fig. 2):

- dysregulated growth suppression,
- resistance to cell death,
- invasion and metastasis,
- aberrant proliferative signaling.

Like other ING family proteins, ING5 is a H3K4me3 sensor [142,143,214], thereby most certainly regulating both, gene silencing and activation. ING5 is also a component of two different HAT complexes [151]. Depending on its catalytic acetyltransferase subunit, i.e. MOZ/MORF or HBO1, it either binds to histone H3 or H4, respectively [148]. As a subunit in the HBOl-MYST-ING-HAT-complex that usually contains BRPF1, ING5 can disassemble BRPF1 from unmethylated H3K4, thereby switching HBO1-specifity from H3 to H4 tails [215]. This suggests a unique role for ING5 as a regulator of HAT affinity to specific histone tails.

ING5 is a histone mark (H3K4me3) sensor and member of the HB01-JADE-hEAF6- and MOZ/MORF-MYST-ING-HAT-complexes, thereby activating target genes by acetylation of histones H3 and H4.

2.10. Target-ING individual cancer epigenomes: possible implications for the ING family proteins in epigenetic cancer therapy

Current multimodal treatment concepts for cancer patients are limited due to toxic side effects in normal cells and frequent development of resistance in cancer cells. Newer strategies target well-defined molecular markers, such as overexpressed Her-2 in breast cancer or Brc-abl fusion in leukemia. They are often effective initially, but fail when subpopulations of resistant cells evolve. Thus, the new paradigm of anti-cancer drug development aims at combating multiple Hallmarks of Cancer simultaneously. In this context, epigenetic-modulating agents harbor a variety of options, since they can (*a*) reverse stem-cell like behavior and chemoresistance and (*b*)simultaneously affect multiple signaling pathways that might be dysregulated in a certain cancer type. Moerover, every patient, thus every individual cancer, has a unique histone code, modification of which is reversible, thereby opening a wide therapeutic window and leaving the genetic code unaffected. Hence, epigenetic "reprogramming" of a cancer cell by either reactivating silenced tumor suppressors or by silencing oncogenes may sensitize cancer cells to lower doses of conventional cytotoxic agents or reverse resistance to other targeted approaches. Indeed, anticancer drugs targeting DNA methyltransferases (DNMTs) and HDACs have demonstrated antitumor activity in the clinic. To date, the best characterized and FDA-approved epigenetic modulating antineoplastic agents are DNMT inhibitors (DNMTi) and HDAC inhibitors (HDACi] for treatment of hematological malignancies. Success in solid tumors has been more elusive, possibly due to ineffective delivery, dosing or scheduling [216]. Hence, different strategies for combining a DNMTi such as 5azaC with other chemotherapeutics like DNA damaging agents or an HDACi, are currently under investigation. Moreover, recent reports on the roles of the bromodomain-containing BET family members and methyltransferase EZH2 in driving cell growth and survival have provided further validation of epigenetic regulators as critical drivers of the transformed cancer cell phenotype [217]. Similar to BET proteins, ING proteins are components of the write-read-erase concept that has been linked with the transfer of epigenetic information. Therefore, they represent both potent biomarkers and a plethora of potential novel targets for future epigenetic cancer therapy. A model for their possible implications is presented in Fig. 3.

2.10.1. Target-ING the mSin3A-HDAC-complex: implications for ING1 and ING2

Cell death and caspase 3-mediated apoptosis in breast cancer cell lines significantly increased when combining 5azaC and the HDACi LBH589 with adenoviral delivery of ING1b [82]. This effect was not significantly dependent on p53-status [89]. It was strongest in estrogene receptor (ER)-negative breast cancer cells, possibly because ING1b can activate transcription of ERα [218,219]. *In vivo*, ING1 plus 5azaC significantly reduced the sizes of subcutaneously xenografted tumors in SCID mice without significant toxicity or development of resistance [89]. These observations suggest a combination of ING1, DNMTi and HDACi as a promising approach for patients with ER-negative breast cancer. In glioblastoma multiforme (GBM), a common and highly malignant brain tumor, downregulation of *ING1* by siRNA resulted in reduced FADD and caspase-3 mediated apoptosis by the HDACi trichostatin A (TSA) [220]. Because neither FADD nor caspase 3 was observed to be a transcriptional target of ING1 [221,222], ING1 function may rather be to stabilize or activate members of this apoptotic pathway. Given the fact that major tumor suppressor pathways are already functionally eliminated in most GBM [223], maintaining ING1 levels in these cells may allow HDACi-induced apoptosis to be of major therapeutic impact. Considering that *ING1* expression is significantly reduced in malignant gliomas [79,80], and that downregulation of *ING1* by siRNA also sensitizes p53-deficient malignant glioma cells to cisplatin-induced cell death [224], ING1 levels in GBM cells might influence the choice of chemotherapy. This strategy may also apply to patients with osteosarcoma, since ING1b

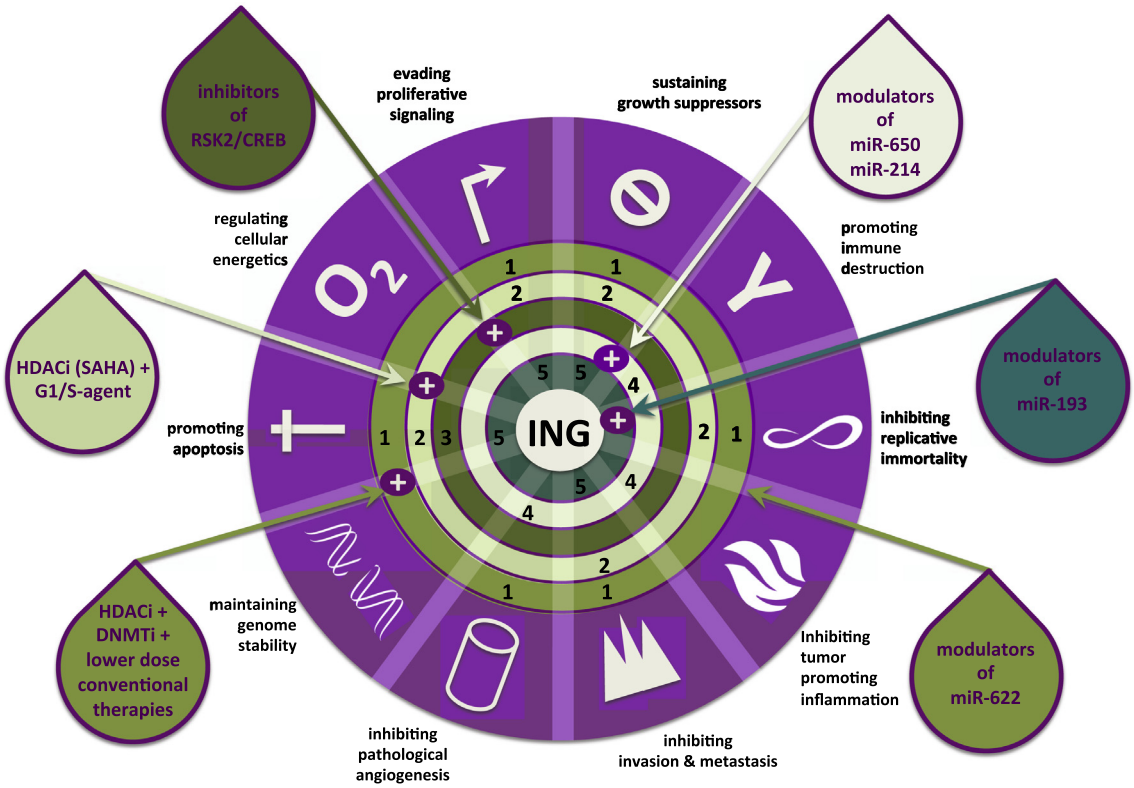

Fig. 3. Balanc-ING the cancer epigenome: ING family proteins as potent therapeutic targets for targeting the Hallmarks of Cancer. *Abbreviations and symbols:* DNMTi – DNA methyltransferase inhibitor, HDACi – histone deacetylase inhibitor, ING (I–5) – inhibitor of growth (1–5), miR – microRNA, RSK2/CREB -serine/threonin kinase RSK2-cycloAMP-response element-binding protein pathway, SAHA - suberoylanilide hydroxamid acid. *For details: see main text.*

showed synergistic effects with DNA-damaging agents and mitotic inhibitors to induce p53-mediated cell death in osteosarcoma cells [225,226]. Considering that *ING1* is downregulated in a variety of common human cancers, including breast cancer and GBM, and that it is a target of miR-622 in gastric cancer, studying the effects of modulating microRNA signatures that may regulate ING1 in other malignancies may represent another step towards individualized cancer therapy.

ING2 is the major molecular target of the HDACi suberoylanilide hydroxamic acid (SAHA) [227], suggesting that targeting of the INGs themselves may prove useful in combination with HDACi and other therapeutic agents that have been shown to induce ING2, thereby promoting p53-acetylation and subsequent apoptosis in cancer cells. This approach may also be effective in cancers upregulating *ING2*, such as gastrointestinal cancers. Intriguingly, *ING2* knockdown by siRNA enhanced growth suppression in adenocarcinoma cells most efficiently when combined with the widely used cytostatic adriamycin, whereas either single agent alone was significantly less effective [122]. As mentioned earlier, *ING2* knockdown suppresses MMP13, thus inhibiting tumor invasion [133,141]. Moreover, *ING2* knockdown sensitizes gastric cancer cells to chemosensitivity with 5-Fluouracil (5-FU) [141]. Therefore, therapy with siRNA against *ING2* may be an approach to patients with ING2-overexpressing tumors. However, methods of siRNA delivery to a targeted location still need to be optimized before going bedside. An interesting project would be to test, whether dose reduction of DNA-damaging agents, but adding SAHA to the drug regimen will result in similar or even higher efficacy. Also, inhibiting H3K4me3 binding by targeting the PHD, using small molecular compounds, may be a way to limit the oncogenic effects of ING2. The H3K4me3 binding region of the PHD forms substantial grooves [115], thereby representing potential targets for small molecules. However, this idea needs further refinement also, in particular with regard to specifity towards the ING2, but not other PHD domains. Since not all ING proteins function as oncogenes, but do share the PHD, targeting this domain may not be broadly beneficial.

2.10.2. Target-ING the MYST-ING3/4/5-HAT complexes

ING3 is frequently downregulated in cancers, particularly in HNSCC and HCC. Moreover, ING3 is negatively regulated by miR-21, a microRNA that is often upregulated in cancers and associated with tumor progression [118]. Hence, modulating miR-21 may increase ING3-expression and subsequently activate antiproliferative genes. In addition, RSK2 signaling through CREB downregulates ING3, thereby shielding HNSCC cells from apoptosis [160]. Thus, selective serine/threonin kinase inhibitors may increase *ING3* transcription and its transcriptionally activating effects.

In addition to various cancer hallmarks affected by all ING family proteins, ING4 is the one that has been reported to additionally affect tumor promoting inflammation and immune destruction. Intriguingly, ING4 has recently been suggested as a prognostic factor in breast cancer based on its ability to negatively regulate NF-κB target gene expression [184]. It also promoted apoptosis in lung cancer cells when co-expressed with Interleukin (IL)-20 [228]. *ING4* is silenced in breast, lung and many other human cancers, where it can promote sensitivity to conventional DNA damaging agents *in vitro* [229–232]. Considering that ING4 is a target of miR-650 and miR-214 in some of these tumors, the possibility of combination therapy with tumor-specific miRNA modulators may be a promising approach.

This concept may also apply to cancers downregulating *ING5*, which is a target of miR-193. Interestingly, *ING5*-downregulation correlated with sensitivity to tamoxifen in ER-positive breast cancer cells in a genome-wide functional RNAi screen [233]. Thus, ING5 might be involved in estrogene signaling in a subset of breast cancer patients. Moreover, ING5 expression levels might serve as a

biomarker to recognize tamoxifen resistant breast cancer phenotypes. Considering, that the HBO1-JADE-HAT complex depends on its association with ING5 in order to enhance DNA replication, blocking ING5 by siRNA might be a future approach for the treatment of cancers overexpressing ING5, such as colorectal cancer.

Concluding Remarks

ING family proteins have proven to be potent targets for epigenetic cancer therapy, particularly in combination with conventional treatments. Their stoichiometric residence in HAT and HDAC complexes and links to DNA methylation make them logical choices for dysregulation of epigenetic status in cancer cells as a means of increasing the therapeutic index of many traditional cancer treatments.

Conflict of interest

None declared.

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